J. Anat. (1976), **121**, 2, pp. 363–370
With 13 *figures*
Printed in Great Britain

The effect of neonatal treatment of male mice with antiandrogens and of females with androgens on the development of the os penis and os clitoridis*

A. GLUCKSMANN,† S. OOKA-SOUDA, E. MIURA-YASUGI AND T. MIZUNO

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo, 113, *Japan*

(*Accepted* 4 *June* 1975)

INTRODUCTION

The morphology of the os penis (baculum) in rodents is known in some detail. Female *mice* of some strains are reported to have a small oval-shaped or slightly elongated os clitoridis (Howard & Migeon, 1962; Hummel, Richardson & Fekete, 1966). Bone is not found in female *rats*, but treatment of newborn animals with testosterones can induce an os clitoridis composed, like the os penis, of a proximal and a distal element; in older animals only the distal element can be induced (Cherry & Glucksmann, 1968; Glucksmann & Cherry, 1972). Castration of 4 day old male mice reduces the size of both parts of the baculum and simultaneous treatment with androgens counteracts this effect (Howard, 1959).

Little information exists about the normal development of the bony structures in the external genitalia of rodents and about the sensitivity of the proximal and distal elements to androgens and antiandrogens. The object of the present study was to investigate these problems in male and female mice.

MATERIAL AND METHODS

Male and female mice of the ICR strain were used in two series of experiments. In series 1, newborn male mice were injected subcutaneously with an antiandrogen, cyproterone acetate (kindly presented by Dr F. Neumann of Schering A.G., Berlin, to Dr I. Lasnitzki). The treatment was started on the day of birth and repeated on the following 3 days. The daily dose was 100 μg in 0·02 ml sesame oil. In series 2, newborn female mice were injected with testosterone (Koch-Light Laboratory, England) or with dihydrotestosterone (Tokyo Kasei Chem.) or with β-oestradiol (Koch-Light Laboratory) on the day of birth and again on the next day at a daily dose of 300 μg in 0·02 ml sesame oil.

The animals were killed at intervals between the day of birth and 180 days. The genital tract was fixed in Bouin's fluid, embedded in paraffin, sectioned at 7 μm and

* Requests for reprints to Professor T. Mizuno, Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113.

† Visiting Professor at the University of Tokyo in 1974. Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge.

stained with toluidine blue (0·01 %, pH 3·4) or Carazzi's haemalum–eosin. Some male and female external genitalia were fixed in ethanol, the baculum and its corresponding structures in the clitoris dissected out, and either stained as whole mounts in toluidine blue to test for the presence of metachromatic cartilage or else photographed with a soft X-ray apparatus (Softex type C-Sm) at 12 kV and 3 mA. At autopsy the ovaries, vaginae and submaxillary glands of females were fixed for histological examination.

Serial sections through the genital tracts of untreated mice at various stages of embryonic and postnatal development were also available for analysis.

RESULTS

The normal development of the os penis and of its corresponding tissue in the clitoris of mice

In late embryos of both sexes, i.e. from about the 16th day of gestation onwards, a formation of closely packed mesenchymatous cells extends parallel to the urethra on its ventral (upper or abdominal) side (Fig. 1). At this stage the cytoplasm and intercellular material are scanty and the latter does not stain metachromatically. No further differentiation occurs in either sex until birth.

Os penis. In males metachromasia appears in the intercellular spaces of the dense mesenchymatous anlage 1 day after birth. The next day bone starts to form in the more peripheral, and cartilage in the more central, parts of the metachromatic region (Fig. 2). The ossification of the outer portion is very rapid and there is no evidence for a cartilaginous precursor of this part of the bone, while towards the base of the penis typical metachromatic cartilage develops which persists until late in life. It is replaced gradually by endochondral ossification (Fig. 3) and is responsible for the growth of the bone which slows down at about 60 days when the cartilage becomes less active, atrophies and finally disappears by about 180 days. Further towards the base of the penis the dense mesenchymatous tissue develops into the

---

All micrographs are taken at a magnification of × 132. The sections reproduced in Figs. 3 and 4 are stained with toluidine blue, all others with haemalum–eosin. In all sections the proximal end of the organs is on the left, the distal on the right, the ventral side at the top and the dorsal at the bottom of the picture. In the skiagrams of Figs. 8, 9, 12 and 13 the reference line measures 10 mm, the distal end is on top and the proximal at the bottom of the photographs.

Fig. 1. Section through the penis of a 16·5 day old embryo showing a condensed cell mass on the ventral side of the urethra. Essentially the same formation is found in sections through the clitoris at this stage of development.

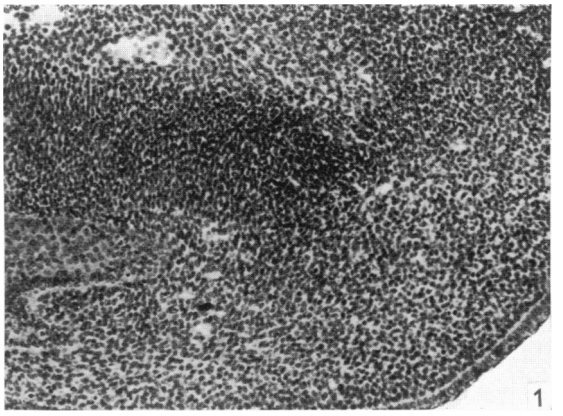
Fig. 2. Section through the penis of a mouse aged 3 days with bone formation on the right and cartilage on the left.

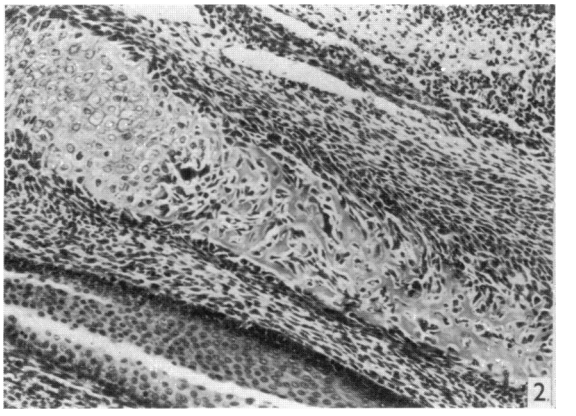
Fig. 3. Section through the base of the baculum of a mouse aged 30 days showing endochondral ossification. As in Fig. 4 the cartilage is stained metachromatically.

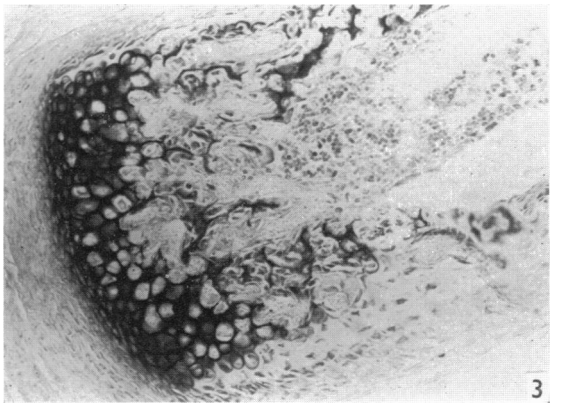
Fig. 4. Section through the distal end of the baculum at the tip of the penis of the same mouse.

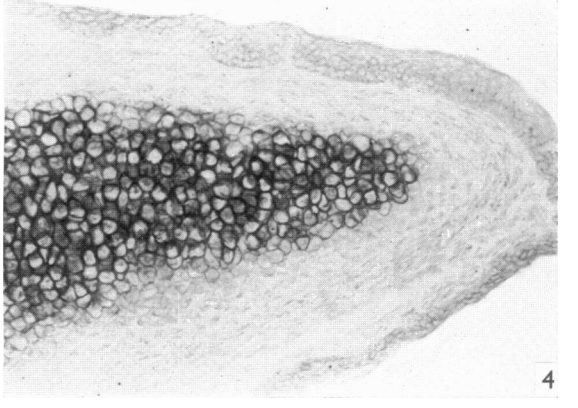
Fig. 5. Section through the clitoris of a mouse aged 3 days showing a small focus of ossification in the dense mesenchymatous tissue. Compare with Figs. 1 and 2.

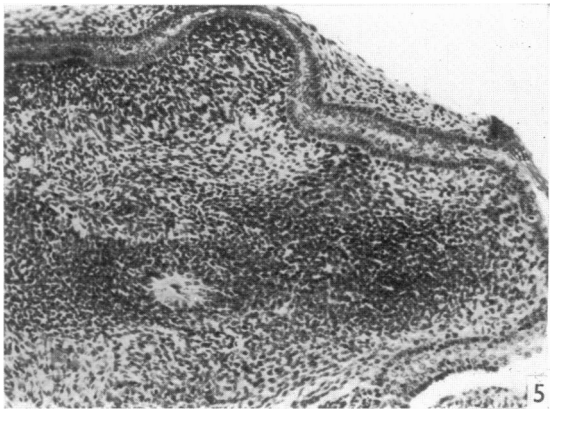
Fig. 6. Section through the clitoris of a mouse aged 150 days showing a small oval os clitoridis. No cartilage is associated with the bone.

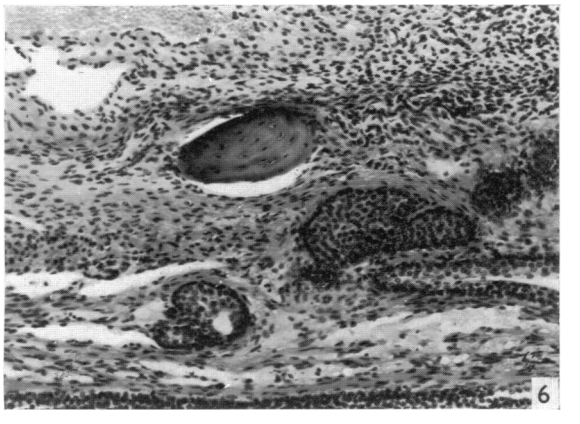












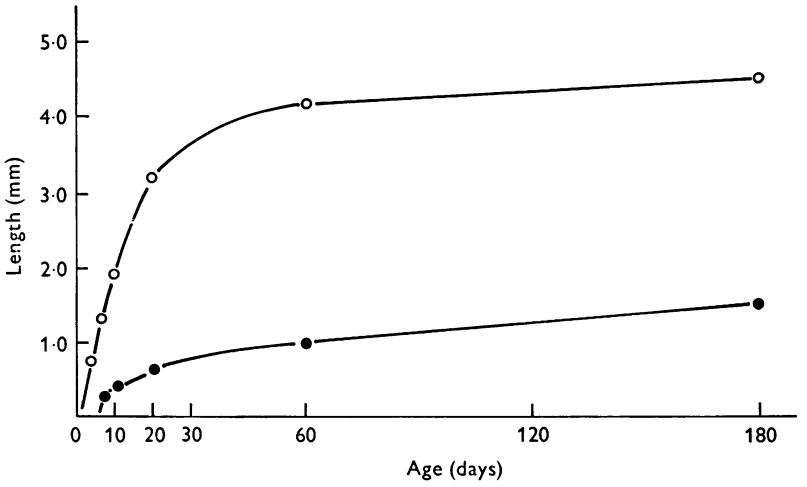


Fig. 7. Growth in length of the proximal (○) and distal (●) elements of the os penis in normal ICR mice.

corpora cavernosa, while towards the tip it differentiates into the distal element of the baculum. Thus the proximal rod of the os penis in mice appears before the distal rod and is formed by direct ossification in its outer and anterior part and by endochondral ossification in its inner and posterior part.

The distal element presents as dense mesenchymatous tissue on day 3 and its outline is clearly defined by day 7. Metachromasia is seen on day 20 and typical cartilage on day 30 (Fig. 4). The tip of the bone forks and is situated under the tip of the glans. The cartilage persists, but gradually calcifies and ossifies.

Fig. 8. Skiagrams of the os penis (*a*) in normal postnatal development (upper row) and (*b*) after neonatal treatment with cyproterone acetate (lower row). (*a*) From left to right: a penis within 24 hours of birth and the dissected bacula of mice aged 3, 7, 10, 20 and 60 days respectively. Note the appearance of the distal rod on day 7 and the progressive calcification of the proximal rod. (*b*) From left to right: neonatally treated baculum on days 7, 10 and 20 respectively. Compare the size and degree of calcification with that of the normal os penis in the row above.

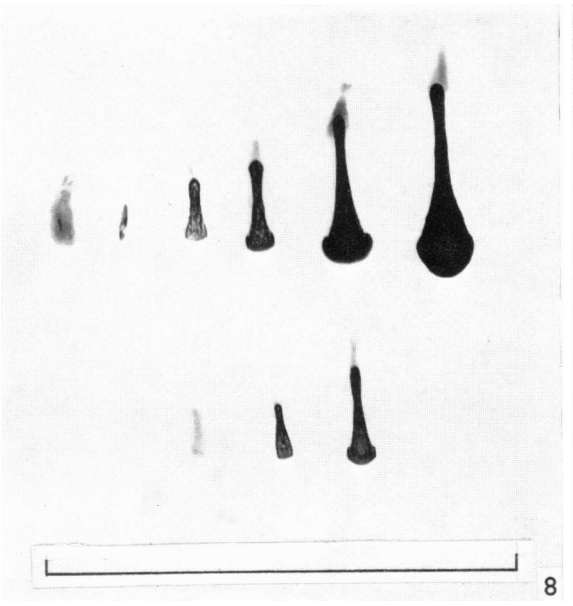
Fig. 9. Skiagrams of the clitoris of mice (*a*) in normal development (upper row) and (*b*) after neonatal treatment with testosterone (lower row). (*a*) From left to right: clitoris of mice aged 1, 5, 10, 15, 20 and 60 days. No bone is discernible. (*b*) From left to right: clitoris of a neonatally treated mouse at 5 days and the dissected os clitoridis of treated mice aged 10, 15 and 20 days. Note the calcification and the absence of a distal element as seen in the penile baculum (Fig. 8).

Fig. 10. Section of the os penis of a 7 day old mouse treated neonatally with cyproterone acetate. The skiagram of this bone is shown on the far left of the lower row of Fig. 8.

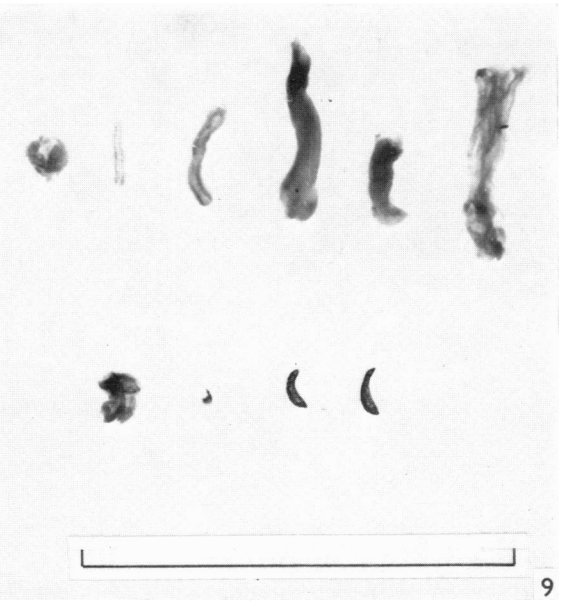
Fig. 11. Section through the os clitoridis of a mouse aged 50 days treated neonatally with testosterone. Compare the size of the bone with that of Fig. 6.

Fig. 12. Skiagrams of the os penis of a normal 10 day old mouse (right) and a neonatally castrated mouse of the same age. Note the forked tip of the normal baculum on the right and the reduced size of the distal element of the castrated mouse (left) with the reduced calcification of the proximal element.

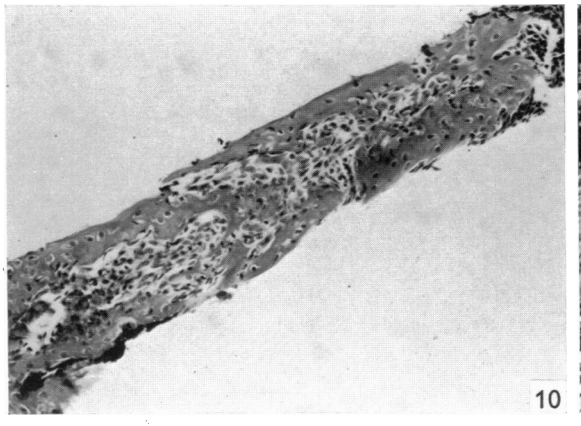
Fig. 13. Skiagrams of the os clitoridis of mice treated neonatally with dihydrotestosterone and aged 23 days (left) and 45 days (right) respectively.



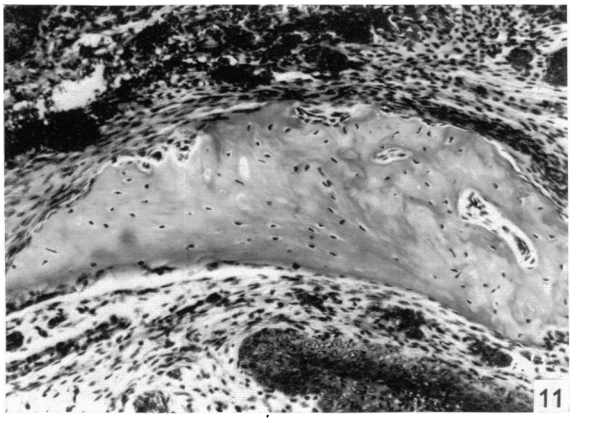
8



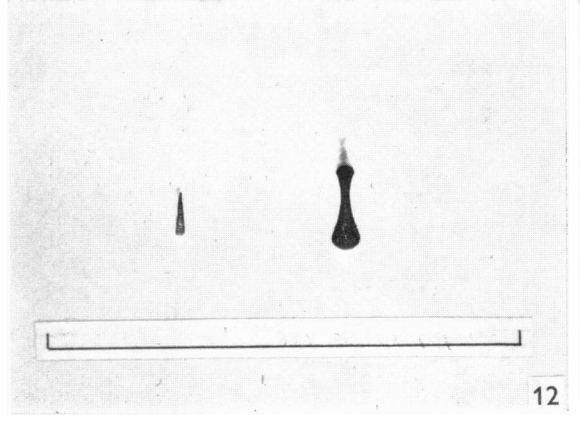
9



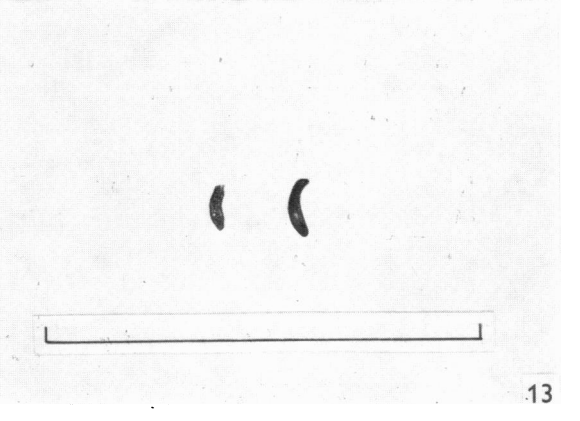
10



11



12



13

The postnatal development of both parts of the baculum is revealed in skiagrams (upper row, Fig. 8). On day 1 (first on the left) a small rod of denser tissue is just discernible in the penis. On day 3 the dissected baculum is more densely calcified in the upper than the lower region of the proximal element, thus indicating the distinction between the direct intramembranous and the endochondral ossifications. By day 7 the distal part of the baculum appears as a shadow, while the proximal part remains denser in the upper than the lower zones and assumes the shape of a 'mandolin'. Calcification and growth of the proximal rod has greatly advanced by 10 days and the distal part is also longer. By 20 days the density to X-rays of the distal component has increased and this has progressed further by 30 days (first on right in upper row, Fig. 8).

The growth rate in length of the two parts of the os penis is plotted in Figure 7. The proximal element grows rapidly up to 30 days and faster than the distal one, but both parts slow down subsequently. Growth of the proximal rod is greatly slowed after 60 days, coinciding with the decreased activity of the cartilage at its base.

Os clitoridis. At birth the dense mesenchymatous tissue in the clitoris resembles in location and extent that seen in the male (Fig. 1). The differentiation of male and female anlage subsequently diverges, as no metachromatic material or cartilage is formed in the female. On day 3 a small acidophilic focus of ossification appears in the female (Fig. 5), which develops into a tiny bone in the adult (Fig. 6).

Skiagrams (Fig. 9, upper row) fail to show up calcified bone in the normal clitoris on the day of birth and on days 5, 10, 15, 20 and 60. In sections a tiny triangular cap of dense connective tissue may represent the distal element of the male baculum, though cartilage is never formed. The os clitoridis may be considered as analogous with that part of the proximal component of the os penis which is formed by direct ossification, since it corresponds with it in development and location.

Effects of treatment with cyproterone acetate on the os penis

Perinatal treatment with the antiandrogen inhibits, but does not suppress, the growth of the os penis (Fig. 8, lower row). Compared with the normal baculum of the same age (Fig. 8, upper row) the increase in length and degree of calcification of the proximal element are reduced and the appearance of the distal element is delayed, though later the distal element differentiates into typical metachromatically staining cartilage. At 7 days the proximal element is osseous (Fig. 10), but hardly calcified (Fig. 8, lower row on extreme left). Castration on the day of birth inhibits the growth of the baculum more than treatment with cyproterone (Fig. 12) and reduces the degree of calcification. A minute triangular cap represents the distal element, which is shown in comparison with the normal control in a 10 day old mouse. Note the forked outer end of the distal rod in the skiagram (Fig. 12, right). The absence of a distal element of the os penis in mice castrated on day 4 and fixed after 40 days (Howard, 1959, Fig. 9) may be an artefact of dissection which is made difficult by the softness of the tissue in the absence of cartilage formation.

Effects of treatment with androgens on bone formation in the clitoris

The effect of neonatal treatment of female mice with testosterone is manifested by a marked increase in the size of the bone (compare Fig. 11 with Fig. 6) and

degree of calcification (Fig. 9). The bone is of Haversian type, formed by direct ossification without a cartilaginous intermediate stage and corresponds to the proximal element of the os penis. The distal rod is represented in sections by a triangular cap of dense connective tissue, but there is no sign of cartilage either histologically or in X-ray pictures. Treatment of neonatal females with dihydrotestosterone produces changes similar to those after treatment with testosterone (Fig. 13). At 23 and 45 days respectively only calcified bone is found, with no evidence of cartilage in the rudimentary distal element. At the inner or posterior end of the proximal rod of the os clitoridis chondrogenesis, even as a preliminary to ossification, is absent.

Androgenic treatment of neonatal females induces persistent oestrus with cornified vaginal epithelium, polycystic ovaries with follicles but no corpora lutea, changes in the female character of the submaxillary gland and absence of cyclical changes in the genital tract. A persistent oestrus is induced also by treatment of newborn female mice with β-oestradiol (Takasugi, 1966), but this does not result in the formation of an os clitoridis. On the other hand, dihydrotestosterone elicits an os clitoridis without inducing persistent oestrus (McDonald & Doughty, 1972). Thus persistent oestrus in itself is not responsible for the induction of an os clitoridis; induction follows androgen treatment, whether oestrus occurs or not.

DISCUSSION

In rats and mice the os penis consists of a proximal osseous part, and a distal ossifying cartilaginous rod. Adult female rats lack, but mice of some strains have, a small os clitoridis in the form of a single element corresponding to the intramembranous osseous part of the proximal element of the male baculum. Neonatal androgenic treatment of female rats induces bone in the clitoris which, like the os penis, consists of a proximal and a distal element; while in mice only a proximal bone is formed (Howard & Migeon, 1962) and the distal element is represented merely by a cap of connective tissue. Treatment of older female rats with androgens elicits the formation of an ossifying cartilaginous distal rod only (Glucksmann & Cherry, 1972). Castration or neonatal treatment of male mice with antiandrogens inhibits growth and calcification of the os penis, particularly of the distal element. Oestrogens fail to promote ossification in the clitoris of mice and rats and do not seem to affect the os penis in adult rats. Progesterones have a slight androgenic action and induce an os clitoridis in mature rats (Glucksmann & Cherry, 1972).

In male and female *mice* the anlage for bone formation in the external genitalia is a formation of closely packed mesenchymatous cells ventral to the urethra. Subsequently cartilage is formed prior to ossification at the base of the proximal rod and (in males only) in the distal rod. No cartilage appears in the female, suggesting that the competence for chondrogenesis is either suppressed or lost early in embryonic development. The absence of cartilage may account for the low growth rate and small size of the bone in the female. It is probably significant that the growth of the proximal part of the os penis, which is faster than that of the distal part, stops when endochondral ossification ceases (Fig. 7). Female *rats* retain competence for cartilage formation in the clitoris at least up to birth, and in the distal rod even in the post-

natal period. The changes in cartilage competence of the dense mesenchymatous tissue of the clitoris in normal development and under the influence of sex steroids are now being investigated in organ culture.

SUMMARY

The os penis in mice and rats is composed of a proximal intramembranous and endochondral osseous element and a distal cartilaginous, ossifying element. Female mice, but not rats, have a small os clitoridis which corresponds to the intramembranous part of the proximal element of the os penis.

In mice of either sex a dense mesenchymatous formation ventral to the urethra is the anlage for the bones of the external genitalia. In the early postnatal period the *proximal* part of the os penis develops as bone at the outer and as cartilage at the basal end of the anlage, while in females a minute focus of ossification differentiates into the small os clitoridis without passing through a cartilaginous phase. The *distal* element of the os penis is formed later than the proximal rod and grows at a slower rate.

Neonatal treatment with an antiandrogen inhibits the increase in size and calcification of the os penis. Neonatal castration is an even more effective inhibitor.

Neonatal treatment with testosterone or dihydrotestosterone, but not with oestradiol, stimulates the growth of the bony proximal os clitoridis, but induces only a rudimentary collagenous distal element.

The differences between mice and rats in the response of the tissues of the clitoris to androgenic treatment are discussed, particularly as regards the differentiation of proximal and distal elements.

A. G. acknowledges the generous support of the Japan Society for the Promotion of Science and the stimulating hospitality of the Zoological Institute, Faculty of Science, University of Tokyo.

REFERENCES

CHERRY, C. P. & GLUCKSMANN, A. (1968). The induction of cervico-vaginal tumours in oestrogenised and androgenised rats. *British Journal of Cancer* **22**, 728–742.

GLUCKSMANN, A. & CHERRY, C. P. (1972). The hormonal induction of an os clitoridis in the neonatal and adult rat. *Journal of Anatomy* **112**, 223–231.

HOWARD, E. (1959). A complementary action of corticosterone and dehydroepiandrosterone on the mouse adrenal, with observations on the reactivity of reproductive tract structures to dehydroepiandrosterone and 11-hydroxyandrostenedione. *Endocrinology* **65**, 785–801.

HOWARD, E. & MIGEON, C. J. (1962). Sex hormone secretion by the adrenal cortex. In *Adrenocortical Hormones* (ed. H. W. Deane). *Handbook of Experimental Pharmacology*, vol. XIV/1, pp. 570–637. Berlin, Heidelberg, New York: Springer.

HUMMEL, K. P., RICHARDSON, F. L. & FEKETE, E. (1966). Anatomy. In *Biology of the Laboratory Mouse*, 2nd edn. (ed. E. L. Green), pp. 247–307. New York: McGraw-Hill.

MCDONALD, P. G. & DOUGHTY, C. (1972). Comparison of the effect of neonatal administration of testosterone and dihydrotestosterone in the female rat. *Journal of Reproduction and Fertility* **30**, 55–62.

TAKASUGI, N. (1966). Persistent changes in vaginal epithelium in mice induced by short-term treatment with estrogen beginning at different early postnatal ages. *Proceedings of the Japan Academy* **42**, 151–155.